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(54) Title: BIOSENSOR DETECTOR ARRAY

(57) Abstract: A method is provided for analysing a sample. The method comprises the steps of: i) contacting the sample with a detector array comprising a plurality of discrete biological sensing elements immobilised onto or within a solid support; wherein each discrete biological sensing element comprises a detectable label whose characteristics change detectably when the element binds to a ligand within the sample; ii) measuring the characteristics of the detectable label for each element of the array to produce a pattern; and iii) performing data analysis of the pattern; wherein the biological sensing elements are capable of binding more than one different ligand.

BIOSENSOR DETECTOR ARRAY

Field of the Invention

The present invention relates to detector arrays comprising biological sensing elements with broad spectrum ligand specificity. These arrays are useful in methods of analysing complex mixtures of ligands such as clinical samples or cell extracts, as well as gaseous or volatile substances of both biological and non-biological origin.

Background to the Invention

The majority of biological detection systems used to date rely on highly specific reactions between detection elements such as antibodies and their target ligands. An example of such an approach is an immunoassay utilising viral peptides to detect antibodies to the virus in human sera. However such systems have a relatively narrow information content since they are designed to recognise only specific reactions between a specific ligand and a specific detection element to generate a positive/negative result. Indeed, great attempts have been made to increase the specificity of such systems and reduce non-specific interactions to reduce the occurrence of false positive and false negative results.

Thus, these highly specific detection systems are capable of generating only a limited amount of information such as the presence or absence of a virus in the sample. To increase the information content of these detection systems requires a large number of properly characterised and highly specific detection elements since there is almost a direct relationship between the amount of information and the number of elements required. Consequently, this approach lacks flexibility and can be expensive if a large number of different detection elements are required.

An alternative strategy mentioned in WO-A-97/49989 is to use sensing elements having less broad specificities. In particular, lectins are exemplified as a suitable type of relaxed specificity element. Since the results obtained from such an analysis are not simple positive/negative determinations. WO-A-97/49989 describes the use of nonlabel detection techniques to generate a pattern that is analysed by comparison with reference samples using, for example, neural network analysis. The nonlabel detection techniques described in WO-A-97/49989 rely on determining an increase in the mass of the sensing element/ligand complex bound to a particular sector on a solid substrate sensor array. These nonlabel detection techniques included surface plasmon resonance (SPR), reflectometry and ellipsometry, although only ellipsometry was exemplified.

However, nonlabel detection techniques require sophisticated apparatus that are difficult to use. WO-A-97/49989 acknowledges that surface mass-based imaging technology was very difficult to use with the biological sensing elements and required the development of a highly specialised protocol for immobilising the elements onto the solid substrate. In addition, the use of lectins is not entirely satisfactory since they only bind carbohydrates.

Consequently, there is a need for an improved, broad specificity, detection system.

Summary of the Invention

The present invention provides an improved method for analysing complex samples using sensing elements having broad specificity for different classes of ligand. Typically, this is achieved by using groups of related biological sensing elements that have been created by modification of a primary element. The detection range (ie. overall binding activity) of such an array of groups of related elements is significantly enhanced compared with the binding activity of the original single element. Furthermore, by contrast to previously described systems, largely uncharacterised sensing elements may be used. In addition, the detection technique used relies on a detectable label attached to the sensing elements, for example a fluorescent label, whose physical characteristics change when the sensing element is bound to a ligand.

Accordingly the present invention provides a method for analysing a sample which method comprises:

(i) contacting the sample with a detector array comprising a plurality of discrete biological sensing elements; wherein each discrete biological sensing element comprises a detectable label whose characteristics change detectably if the element binds to a ligand within the sample;

(ii) measuring the characteristics of the detectable label for each element of the array to produce a pattern; and

(iii) performing data analysis of the pattern;

wherein the biological sensing elements are capable of interacting (typically binding) with more than one different ligand.

Typically, the discrete biological sensing elements are immobilised onto or within a solid support.

This method is suitable for analysing a sample of a complex mixture of ligands. The term analysing a sample of complex ligands has its natural meaning. However, it will be understood that the method may be advantageously applied to simple mixtures of ligands, or to samples comprising single species of ligand, or any combination thereof. Furthermore, the analysing step of this method will be understood to include the profiling and/or fingerprinting and/or reference point embodiments as described herein.

The sample usually comprises a complex mixture of ligands. The sample may be a biological or non-biological sample, or mixtures or combinations thereof.

The detectable label is preferably a fluorescent label. The change in the characteristics of the label is typically detected by optical or electrical means, for example a change in emission intensity, excitation or emission wavelength, excited state lifetime and/or polarization. Preferably, the detection is via optical means.

The biological sensing element is preferably a protein, such as a protein selected from bacterial periplasmic binding proteins, membrane proteins, enzymatic proteins, odorant binding proteins from mammalian or insect olfactory organs and DNA binding proteins. Preferably, the protein is not a lectin or an antibody.

The complex mixture of ligands may comprise volatile and/or non-volatile compounds. Alternatively, the ligands may be present in, for example on the surface of, pathogenic organisms such as clinically important compounds or viruses.

Data analysis of the pattern obtained in step (ii) above is preferably performed by comparing the pattern obtained with a database of patterns from one or more reference samples.

According to a broad aspect, the present invention relates to a method for fingerprinting a sample comprising determining the activity profile of a sample against two or more different entities wherein said entities have a broad specificity binding activity.

The present invention also provides a fingerprint, wherein said fingerprint is obtained from said method for fingerprinting a sample.

The present invention also encompasses a sample when analysed by said methods of the present invention.

The present invention also provides a detector array comprising a plurality of discrete biological sensing elements (typically these will be immobilised onto or within a solid support) wherein each discrete biological sensing element comprises a detectable label whose characteristics change detectably when the element binds to a ligand within the sample; and wherein the sensing elements are provided in groups, each group comprising a biological sensing element and at least one variant of said element, said variant differing from the element in its ligand binding specificity and/or affinity. Typically, the specificity and/or affinity of the variant does not need to be known whereas the specificity of the element is usually at least partially characterised. This is an advantage of the prsent invention.

The biological sensing elements are preferably proteins, such as proteins selected from bacterial periplasmic binding proteins, membrane proteins, odorant binding proteins from mammalian or insect olfactory organs and DNA binding proteins. Preferably, the proteins are not lectins or antibodies.

The variants in each group in the array are typically obtained by modifying a primary element in the group to alter its binding specificity and/or affinity. For example, the variant may be derived from one of the other biological sensing elements by chemical modification. Alternatively, the variant is derived from one of the other biological sensing elements by mutagenesis, which may for example, have been carried out by site-directed mutagenesis of a ligand binding site and/or replacing a fragment of the biological sensing element polypeptide with a different polypeptide fragment. Variants may even be prepared using combinations of these techniques.

The arrays of the present applications have a wide variety of uses in analysing samples. In one embodiment, the present invention provides the use of an array of the invention in a method of detecting clinically important compounds in a liquid or substantially gaseous sample.

In another embodiment, the present invention provides the use of an array of the invention in a method of detecting volatile compounds in a substantially gaseous sample.

Detailed Description of the Invention

Although in general the techniques mentioned herein are well known in the art, reference may be made in particular to Sambrook *et al.*, Molecular Cloning, A Laboratory Manual (1989) and Ausubel *et al.*, Short Protocols in Molecular Biology (1999) 4th Ed, John Wiley & Sons, Inc. In addition, references relevant to general array methodology include Chu *et al.*, 1994; Ekins, 1996; Ekins *et al.*, 1990; and Ekins *et al.*, 1990.

A. Biological sensing elements

The term biological sensing element is intended to mean a biological molecule, typically a polypeptide, that has the ability to bind to another molecule, termed a ligand, usually in a reversible manner. Thus, for example, antibodies are biological sensing elements, as are cell surface receptors. The term "biological" refers to the nature of the sensing element and not the nature of the ligands to which it binds, which may be either biological, such as pathogen-derived proteins, or non-biological, such as petrochemicals.

The term "discrete" in relation to the biological sensing elements means that each element is placed on or in the detector array such that the spacing between each element allows each individual element to be resolved by the detection equipment.

The sensing elements are typically proteins/polypeptides. Preferably said proteins have broad specificity, which is explained in more detail below.

Preferably sensing elements are proteins/polypeptides which are of small size. Preferably small size means less than 100kDa, preferably less than 70 kDa, more preferably less than 60kDa. In a most preferred embodiment, sensing elements are proteins/polypeptides which are 50kDa or less in size. These sizes may be estimated using polyacrylamide gel electrophoresis, or may be calculated molecular weights. These sizes may exclude fluorophores or attached groups, and relate to the size of the polypeptide core of the sensing element.

Preferably the sensing elements are proteins/polypeptides which can be expressed (typically these are readily over-expressed) in a suitable host organism, such as a micro-organism, typically *E.coli*. This is a standard procedure well known to those skilled in the art, and is discussed in more detail herein below. Briefly,

nucleic acid encoding the polypeptide is cloned into an expression vector and this expression vector in transformed into a host strain of *E.coli* for protein expression. Expression is induced, and preferably proteins/polypeptides suitable for use as sensing elements of the present invention are highly expressed, and preferably readily extracted or purified as discussed in the Examples section. Less preferred are polypeptides which form insoluble inclusion bodies on expression, and require more complicated extraction techniques.

Preferably sensing elements are proteins/polypeptides which do not comprise CYS residues, or can tolerate the mutagenic removal and/or relocation of said CYS residues without destroying their activity and/or properties. A CYS residue is typically introduced into the proteins/polypeptides of the present invention for fluorophore attachment to facilitate their use as sensing elements. If a polypeptide naturally posesses a CYS residue, there is the possibility that this will be suitable for fluorophore attachment. However, fluorophore attachment may be better carried out at a site chosen by the person working the invention, for example to produce a superior ligand-dependent change in fluorescence, or to place the fluorophore nearer or farther away from a ligand binding site in order to optimise the behaviour of the sensing element. It is therefore preferred that a polypeptide for use as a sensing element in the present invention has no CYS residue and can tolerate one being introduced, or may have any existing CYS residue(s) removed or relocated, without adversely affecting its ligand binding activity.

Suitable location(s) in protein/polypeptide sensing elements for the introduction of CYS residues for fluorescent labelling may be chosen by a person skilled in the art, preferably placing them so that they do not interfere with the binding site (if known). Preferably placed on or near residues which move and/or change conformation on ligand binding. Preferably placed at a location which will not interfere with expression/purification/immobilisation of the polypeptide sensing element. Preferably placed at a location whose exposure to solvent is altered (eg increases or decreases) in response to ligand binding. Preferably placed in accordance with any other considerations which may vary according to the particular needs of the person working the invention. In a highly preferred embodiment, each of these considerations is met in placement of the CYS residue for fluorophore labelling. If sufficient information is not available to make meaningful choices about the placement of the CYS residue(s) *a priori*, a simple trial-and-error approach may be used, making a number of variants and picking the variant with the CYS location resulting in the sensor element with the most suitable characteristics as described herein.

Preferred sensing elements are proteins/polypeptides which may be tagged for purification/immobilisation without such tagging adversely affecting the binding activity of the polypeptide. Exemplary tagging systems include the 6his tag as is well known in the art and described herein. Preferably polypeptide sensing elements according to the present invention may be tagged at the N-terminus, the C-terminus, or even both or other location(s) within the polypeptide chain, preferably such tagging does not compromise the binding activity of the sensor polypeptide according to the invention.

Preferred sensing elements of the present invention are proteins/polypeptides which, when labelled with a CYS-attached fluorophore exhibit a ligand-dependent change in fluorescence. A ligand dependent change in

fluorescence may be a change in intensity of fluorescence, a change in emission wavelength of fluorescence, a change in absorption characteristics of the fluor or any other measurable characteristic of the fluorescence, which characteristic is changed, altered (eg. increased or decreased), modulated or otherwise affected by binding of a ligand.

Some proteins intrinsically possess broad specificity. Examples of such proteins include olfactory binding proteins (OBPs), major urinary proteins (MUP). These and other polypeptides may have their specificity altered by mutation of the polypeptide, in particular of the binding site. Altered may mean broadened (also termed 'relaxed') specificity or may mean narrowed specificity, or may mean changed specificity in the sense that a different set of ligands may bind to the altered polypeptide, whether or not this new set of ligands is a subset, a superset, or a non-overlapping set of ligands with respect to the ligand-binding characteristics of the unaltered polypeptide. It is a preferred characteristic of the polypeptide sensors of the present invention that they tolerate alterations such as mutations to their binding site(s), as discussed under the term 'variants' herein. By 'tolerate these alterations', it is meant that polypeptides of the invention are preferred if they can be altered and/or mutated as described herein without destroying the activity of the protein, for example by causing misfolding, insolubility, or loss of function of one or more of the preferred characteristics as discussed herein.

It will be understood that candidate sensor polypeptides according to the present invention are not restricted to comprising full length naturally occurring polypeptides. Fragments, truncations, domains (whether singly or in combination) or concatenations of such molecules may be utilised. Futhermore, any of these may be altered, mutated or modified to produce variants as described herein, such as variants with relaxed (broadened) specificity. Artificial polypeptides may be employed, or may be combined with naturally occurring and/or altered polypeptides as described. In one embodiment, candidate polypeptide molecules having a lipocalin fold may be employed.

Exemplary polypeptides useful as sensors in the present invention include periplasmic binding proteins, olfactory binding proteins, membrane proteins, DNA binding proteins, maltose binding proteins, phosphate binding proteins, glucose-galactose binding proteins, arabinose binding proteins, glutamine binding proteins and others. Other molecules may be employed as sensors in the present invention, such as avidin. Avidin is an example of a sensor molecule for use in the present invention which may be used even when randomly labelled with fluorophore, whereas the polypeptides discussed herein are typically specifically labelled with fluorophore through cysteine residues.

Combinations of polypeptide and non-polypeptide molecules may be employed as sensors in the present invention, such as maltose binding protein complexed with cyclodextrin. This combination is particularly advantageous in the study of non-steroidal anti-inflammatory compounds as ligands.

Binding characteristics of the polypeptide based sensors of the present invention may be investigated using one or more test ligand(s). These may comprise a panel of ligands or may comprise one or a number of

candidate molecules binding a particular polypeptide sensor. In the case of bOBP, an example of a suitable test ligand is thymol. Other bOBP test ligands include camphor, decane, or any other analyte of interest. In the case of maltose binding protein, suitable ligands include maltose, cyclodextrin. In the case of a maltose-cyclodextrin combination used as a sensor, suitable ligands include non-steroidal anti-inflammatory compounds.

Exemplary proteins discussed herein are particularly suitable sensing elements of the present invention. For example, olfactory binding proteins intrinsically posess broad specificity, are of relatively small size, (are less than 50kDa), are readily over-expressed in *E.coli*, accept the introduction of CYS residue and attachment of a fluorophore thereto without destroying their binding activity, tolerate the addition of suitable tag for purification such as the 6his tag, exhibit ligand-dependent changes in fluorescence, and are able to accommodate mutations to their binding site(s) without destroying the above-described properties. OBPs are therefore preferred sensing elements. Highly preferred are bovine OBPs (bOBPs).

Exemplary sensing elements may include proteins selected from bacterial periplasmic binding proteins, membrane proteins, odorant binding proteins from mammalian or insect olfactory organs and DNA binding proteins. Preferably, the proteins are not lectins and/or preferably the proteins are not antibodies. A particularly preferred sensing element is the odorant binding protein from cows.

It may not necessary to use the entire protein, instead, it may be possible to use only that fragment of the protein which contains the ligand binding site, together with sequences required to maintain the conformation of the binding site if necessary (e.g. if the amino acids that constitute the binding site are brought into proximity with each other by protein folding).

The biological sensing elements are linked to a detectable label such that when the sensing elements bind a ligand, there is detectable change in a characteristic of the label, such as a change in a fluorescent property, for example intensity, excited state lifetime, excitation or emission wavelength or polarisation. Preferably the label is a fluorescent group with excitation and/or emission wavelength in the optical spectrum (350 to 750 nm). More preferably the label shows an increase in emission intensity and/or a shift in emission wavelength.

Examples of fluorescent proteins which vary among themselves in excitation and emission maxima are listed in Table 1 of WO 97/28261. These (each followed by [excitation max./emission max.] wavelengths expressed in nanometers) include wild-type Green Fluorescent Protein [395(475)/508] and the cloned mutant of Green Fluorescent Protein variants P4 [383/447], P4-3 [381/445], W7 [433(453)/475(501)], W2 [432(453)/480], S65T [489/511], P4-1 [504(396)/480], S65A [471/504], S65C [479/507], S65L [484/510], Y66F [360/442], Y66W [458/480], I0c [513/527], W1B [432(453)/476(503)], Emerald [487/508] and Sapphire [395/511]. This list is not exhaustive of fluorescent proteins known in the art; additional examples are found in the Genbank and SwissProt public databases.

Alernatively, fluorophores such as fluorescent dyes may be used. Examples of fluorescent dyes include the following non-limiting list of chemical fluorophores of use in the invention, along with their excitation and emission wavelengths, as presented in Table 1.

Table 1

Fluorophore	Excitation (nm)	Emission (nm)	Colour
PKH2	490	504	green
PKH67	490	502	green
Fluorescein (FITC)	495	525	green
Hoechst 33258	360	470	blue
R-Phycoerythrin (PE)	488	578	orange-red
Rhodamine (TRITC)	552	570	red
Quantum Red™	488	670	red
PKH26	551	567	red
Texas Red	596	620	red
Cy3	552	570	red
Iodoacetylnitrobenzoxadiazole	482	520	green
Acrylodans	360	430-550	colourless

Preferred fluorescent dyes are iodoacetylnitrobenzoxadiazole and acrylodans.

Fluorophores are preferably linked to the biological sensing elements via a cysteine residue on the biological sensing elements. The cysteine residue is introduced by mutagenesis at a position where the attached dye shows a change in its fluorescence properties upon ligand binding.

In an especially preferred embodiment, the sensing elements are linked to an affinity tag such as a hexahistidine or glutatione-S-transferase sequences such that the sensing elements can be easily immobilised on a solid matrix via the affinity tag and its ligand (for example Ni-NTA or glutathione). Where the sensing element is a polypeptide, it is particularly preferred that the sensing element is encoded by a polynucleotide which encoded the sensing element linked in frame to an affinity tag present at the C-terminus of the sensing element. This has the advantage that during mutagenesis procedures, any mutations that result in premature termination or frame shifts will lead to sensing elements lacking the C-terminal affinity tag. Such mutants will not then bind to a solid matrix via the relevant ligand and may easily be distinguished from full length mutant sensing elements.

The biological sensing elements used typically have broad specificity and preferably bind to varying extents a wide variety of ligands. Preferably, the sensing elements are capable of binding a broad range of structurally diverse ligands. Thus, typically, the sensing elements bind to more than one ligand, by contrast to the highly specific elements used in other detector arrays. Although not every sensing element in the array need have broad specificity, it is preferred that substantially all the elements have broad specificity. Broad

specificity may also be understood to relate to the structural determinants of the ligand(s) to which the sensing elements bind. A broad specificity sensing element preferably binds a number of ligands, which ligands may apparently lack a common structural determinant such as a conserved functional group(s) or particular size or shape of molecule, which shared features by contrast usually define classes or types of molecules (ligands) binding to highly specific sensing elements used in other arrays. An example of this characteristic may be found in porcine olfactory binding protein (pOBP). This is an example of a polypeptide sensing element useful in the present invention. This protein has broad specificity and as such is a preferred sensing element. Ligands known to bind to pOBP include benzophenone, benzylbenzoate, dihydromyrcenol, IBMP, selenazol, thymol and undecanal. Clearly, a comparison of the chemical structures of these diverse compounds illistrates the meaning of the term broad specificity. Furthermore, an exemplary characteristic of the term broad specificity as explained above is the lack of common chemical and/or structural features between the various ligands which bind to the broad specificity element. This characteristic is clearly demonstrated in the example of pOBP, since other than the most basic structural feature of being organic molecules, there is no common structural determinant between the various ligands binding pOBP.

The character of the interaction between ligand and sensing element can be a further or an alternative indicator of the broad specificity of the sensing element in that binding to broad specificity elements of the present invention is not generally of the 'lock and key' type associated with for example an enzyme-substrate interaction which often demands a very precise spatial fit of the ligand (eg substrate) into the element (eg the enzyme molecule). The mechanism of binding of broad specificity sensing elements of the present invention to the ligands is preferably less rigid, and resembles a dissolution of the ligand into the binding site rather than a fixed three-dimensional co-ordination of particular chemical groups of the ligand. Thus, the interaction of ligand with a broad specificity sensing element of the present invention is less likely to be absolutely dependent on a particular feature of the sensing element, such as a particular amino acid residue, but is more likely to be affected to a smaller degree by such mutations, for example by a change in binding affinity or a change in the profile of ligands to which it will bind, rather than an absolute abolition (or restoration) of binding by a single mutation as can be found in other arrays, for example receptor-ligand interactions.

A broad specificity sensing element of the present invention is thus less likely to bind its various ligands through hydrogen bonding, salt bridges and the like, but is more likely to retain the ligand through energetic considerations such as entropy and/or water displacement or via a larger number of weaker bonding forces such as electrostatic forces and/or van-der-waals and/or hydrophobic-hydrophilic style interaction as contrasted with, for example the strong hydrogen bonding exhibited by other ligand-sensor interactions.

A broad specificity sensing element of the present invention is less likely to have a binding site which is precisely defined with respect to amino acid residues which may co-ordinate ligand binding, but is more likely to have a binding site which is less specifically defined, or may be defined geometrically (eg. defined as an area or surface or pocket on the polypeptide rather than defined chemically by reference to particular

amino acid residues).

With respect to the variety of ligands that each element can bind, it is preferred that at least one of the elements, or groups of elements, can bind ligands from more than one class of compounds. By classes of compounds, we mean chemically distinct groups of chemical compounds, such as polypeptides, lipids, carbohydrates, aliphatic, aromatic and heterocyclic compounds.

An advantageous feature of the arrays of the present invention is that the biological sensing elements may be organised into groups (although not necessarily in terms of physical proximity in the array) of related molecules. In particular, the groups comprise biological sensing elements that are variants of one another. Although not every member of the group need be a variant of one of the other members of the group, it is preferred that substantially all members of the group are variants.

A group typically comprises at least two members, preferably at least three or four members. Of these, at least one member is a variant of at least one other member.

The term variant means that a member is derivable from another member. For example, a member may be chemically modified to produce another member. The chemical modification will typically result in a change in the structure of the biological sensing element such that its specificity and/or affinity have been altered. Variants may be obtained using standard techniques such as chemical modification and/or biological mutagenesis techniques. Chemical modification may be effected using reagents known in the art.

Mutagenesis techniques include site-directed mutagenesis of the ligand binding site or any other part of the biological sensing element that results in a structural change affected the binding specificity and/or affinity of the variant. Alternative techniques include domain swapping, whereby using standard cloning technology, sections of an element are replaced with sections from a related or unrelated polypeptide. Mutagenesis includes insertions, deletions and substitutions. Amino acids may be non-naturally occurring amino acids to increase the structural diversity.

A particularly preferred method for mutagenising biological sensing elements is to amplify the gene for the biological sensing elements by the polymerase chain reaction under conditions where there are random mistakes made in the nucleotides being incorparated. The conditions under which such 'error prone pcr' occurs are well known to those skilled in the art. The mixture of randomly mutated genes is then inserted into an approriate vector, transformed into a host and followed by screening the resulting bacteria or viruses using standard techniques (such as expression screening or phage display). The polynucleotide encoding the sensing element conveniently also encodes a reporter fusion protein in frame with the sensing element construct to allow easy identification of the mutagenised proteins over other bacterial/viral proteins. As discussed above,the polynucleotide encoding the sensing element comprises a sequence encoding an affinity tag such that the affinity tag is produced in frame at the C-terminus of the sensing element.

Mutagenised proteins may be purified directly from the bacteria/viruses or the polynucleotide constructs obtained and cloned into other suitable vectors/host for expression and purification of the biological sensing elements.

B. Arrays

Typically, the biological sensing elements are immobilised onto or in discrete regions of a solid substrate. The substrate may be porous to allow immobilisation within the substrate or substantially non-porous, in which case the sensing elements are typically immobilised on the surface of the substrate. The solid substrate may be made of any material to which polypeptides can bind, either directly or indirectly. Examples of suitable solid substrates include flat glass, silicon wafers, mica, ceramics and organic polymers such as plastics, including polystyrene and polymethacrylate. It may also be possible to use semi-permeable membranes such as nitrocellulose or nylon membranes, which are widely available. The semi-permeable membranes may be mounted on a more robust solid surface such as glass. The surfaces may optionally be coated with a layer of metal, such as gold, platinum or other transition metal. A particular example of a suitable solid substrate is the commercially available surface modified glass microscope slides (Xenopore Inc.).

The solid substrate is conveniently divided up into sections. This may be achieved by techniques such as photoetching or by the application of hydrophobic inks, for example teflon-based inks (Cel-line, USA).

Attachment of the sensing elements to the substrate may be by covalent or non-covalent means. Typically, the sensing elements are attached to the substrate via a layer of molecules to which the sensing elements bind. For example, the sensing elements may be labelled with biotin and the substrate coated with avidin and/or streptavidin. A convenient feature of using biotinylated sensing elements is that the efficiency of coupling to the solid substrate can be determined easily. Since the sensing elements may bind only poorly to some solid substrates, it is often necessary to provide a chemical interface between the solid substrate (such as in the case of glass) and the sensing element. Examples of suitable chemical interfaces include organofunctional silanes and long-chain thiol alkanes with terminal activatable groups such as terminal carboxylic acid groups. Another example is the use of polylysine coated glass, the polylysine then being chemically modified using standard procedures to introduce an affinity ligand such as nitrilotriacetate (NTA). Other methods for attaching molecules to the surfaces of sensor chips by the use of coupling agents are known in the art, see for example WO98/49557.

It is desirable to confirm the efficiency of coupling using standard techniques to establish the amount of sensing element bound at each cell on the solid substrate. This information may be used to normalise the results obtained from various positions in the array.

C. Assay procedure

Samples are tested by contacting the sample with the sensor array under specific conditions and then measuring the change in the characteristics of each element of the array. One advantage of the assay of the present invention is that by contrast to other methods, it is not necessary to remove unbound constituents by washing prior to making a measurement. This also allows either kinetic (time dependent) or equilibrium (time independent) measurements to be made

The conditions under which the sample is contacted with the array may be varied to achieve further diversity in the specificity and/or affinity characteristics of the sensing elements (for example the use of different pH and/or salt concentrations).

Samples may be in gaseous, liquid or solid form (or combinations thereof) such as in the form of solid samples, gaseous samples extracted from the atmosphere, liquid environmental samples (for example from a contaminated site), gaseous biological samples such as exhaled air or liquid biological samples such as saliva, blood, serum, sweat, urine, milk, bone marrow, cerebrospinal fluid, synovial fluid, amniotic fluid or lymphatic fluid. Solid samples may be processed in suitable solvent, such as water or organic solvents, to produce liquid samples. Solid samples may also be pyrolysed to produce gaseous samples.

The complete sensor array is typically read by charged coupled device (CCD) camera or confocal imaging system. Alternatively, the sensor array may be placed for detection in a suitable apparatus that can move in an x-y direction, such as a plate reader. In this way, the change in characteristics for each labelled sensor element can be measured automatically by computer controlled movement of the array to place each discrete element in turn in line with the detection means.

The detection means are capable of interrogating each sensor element optically or electrically. Examples of suitable detection means include CCD cameras or confocal imaging systems.

The results obtained for a given array with a given sample under given conditions are termed a "pattern". The pattern will generally be in the form of numerical values for each discrete element, such as in the form of a matrix, table or other data array (which may be one, two or three-dimensional. The results obtained may also be in a non-numerical form such as a graphical representation (several detection methods such as spectrometry give rise to results presented as graphs) but these are preferably capable of being quantitated to provide numerical values. The use of CCD will usually result in an image made up of discrete pixels with a grey-scale or colour intensity for each pixel. The pixel values are numerical data but may be displayed as grey-scale or colour images.

To interpret the pattern, the pattern is subjected to statistical analysis by comparison with a reference database. The reference database may be generated by analysing samples of known origin, and usually known constituents, by the method of the invention to produce a pattern for each sample. These are then

stored by electronic means to generate a reference library of patterns. The pattern obtained for an unknown sample is entered into suitable computer software and a best-fit obtained. The software may also indicate the degree of statistical certainty with which the best-fit match has been made and optionally set a threshold where a sample is rejected as unknown. These techniques are well-known in the art.

The use of neural net-type software is advantageous since the system can be "trained" to improve its ability to discriminate between samples.

D. Applications

The methods and arrays of the present invention may be used in a variety of different applications, such as identifying particular compounds or groups of compounds in a sample. For example, they may be used to detect pathogens, such as bacteria, fungi or viruses, in environmental or biological samples. They may be used to detect molecules associated with and/or indicative of pathological states. They may also be used to detect chemical contamination in environmental samples such as air or water.

Thus the methods and arrays of the present invention may be used in a variety of industrial, clinical and environmental applications.

The sensor arrays of the present invention may be provided as kits. Such kits will typically comprise at least one sensor array and optionally reagents required for standardising reaction conditions, such as buffers. The kit may also comprise the detection means and/or analysis software, optionally comprising a database of reference sample patterns. The kit will also generally comprise instructions for using the kit.

It is an advantage of the present invention that the fluorescent group is an integral part of the individual sensing components of the array. This allows a more streamlined and/or labour-saving process to be used in the analysis, alleviating the need for a fluorescent dying stage to be performed after the ligand binding stage of the procedure.

The present invention will now be illustrated by means of the following examples which are illustrative only and not limiting.

Description of the Figures

Figure 1 is a diagram showing a strategy for producing and using an array of the invention.
Figure 2 is a graph showing Fluorescence Emission Spectra of immobilised bOBP labeled with acrylodans following the addition of thymol or menthol.
Figure 3 is a bar graph.

EXAMPLES

Example 1 - Generating an array of variants of the maltose binding protein (mbp)

A nucleotide sequence encoding maltose binding protein (*malE* gene) is cloned into an expression vector in frame with a C-terminal hexahistidine sequence (6xHis tag). The vector is the commercially available pET28b. The *malE* gene carries the signal peptide sequence to ensure periplasmic expression. In addition the mbp coding sequence has a mutation (for example at position 337 in the protein sequence) so that the protein has a cysteine residue.

Error prone PCR or cassette mutagenesis with mixed mutagenic primers is used to produce variants of the *malE* gene using the vector DNA as a template. These methods are well known in the art.

The mixture of randomly mutated DNA molecules is transformed into a suitable host strain (such as *E. coli* BL21 (DE3)). Transformation conditions are chosen so that each cell takes up a maximum of one molecule of DNA. The cells are then plated onto nutrient agar (containing an antibiotic for selecting only those cells which have been transformed) in a Petri dish and left to grow overnight.

Individual colonies are picked and inoculated into the wells of a microtitre plate. The cells are left to grow for a few (typically 4) hours and then half the contents of each well transferred to a new microtitre plate (the 'master' plate). Protein expression is induced in one of the plates and the other is stored.

After a further period of time (typically more than 2 and less than 24 hours) the cells are centrifuged in the plates and the supernatant liquid removed. The cells are then osmotically shocked to release the contents of the periplasm. The cells are then centrifuged and the supernatant transferred to a new microtitre plate.

A fluorescent dye, such as iodoacetylnitrobenzoxadiazole, is then added to each well of the microtitre plate to label the protein via a reaction with a cysteine residue present in the protein.

A microscope slide coated with poly(lysine) is chemically modified using published procedures to introduce nitrilotriacetate groups. These are then converted to the nickel or copper complex by adding a solution of the sulphate salt of the respective metal ion.

The slide is then washed and the contents of each well are then spotted onto the modified microscope slide and allowed to react in a humid atmosphere until binding has reached equilibrium. The slide is then washed again and is now ready for use.

Example 2 - Using an array of fluorescent proteins

A slide carrying an array of fluorescent proteins as described in Example 1 is mounted in a flow cell such that solution comprising test compounds can be passed over its surface.

Four or more different compounds are tested with the array, either individually or in various combinations.

To measure the pattern for each compound or combination, the flow cell is placed on the stage of a fluorescence microscope so that it is excited by light of a wavelength that causes fluorescence of the dye attached to the sensing elements.

An image of the fluorescent light emitted from the array is collected before and after exposing the slide to a sample for analysis.

A comparison of the patterns of fluorescence before and after exposure of the slide to the samples that contain only one compound is used to initiate training of a neural net implemented in software. Once the net has been trained, the patterns obtained for combinations of compounds are fed into the neural net to determine whether the net can discriminate individual compounds present either singly or in a mixture. Finally, a blind study is conducted using samples containing various combinations that are not known in advance to the tester. The tester feeds the patterns obtained into the neural net software and compares the answer provided with the actual known sample composition.

Example 3- Immobilisation of Site Specifically Labeled Bovine Odorant Binding Protein onto Microtitre Plates

A nucleotide sequence encoding the bovine odorant binding protein (bOBP) and carrying silent mutations that optimise codon usage in the expression host (*Escherichia coli*) is cloned in to the expression vector pET24a using techniques well known to those skilled in the art. The sequence of the gene is shown as SEQ. ID. No.1.

Mutations are made at positions 36 and 89 in the protein sequence using the technique of polymerase chain reaction according to the following method.

1. Prepare template. The bOBP gene is subcloned in the pBluescript vector using standard molecular biology techniques well known to those versed in the art.
This plasmid construct is then used as the template for an inverse PCR.

2.The primer set is designed such that 5' ends of two primers are adjacent to each other. There is no overlap or gap between the two ends.
The primers contain the necessary sequence mismatch(es) to introduce one or more base mutations. The mutations may be either to introduce a cysteine residue for the purposes of labelling the bOBP with a fluorophore or to change residues in the ligand binding site.

3.Using turbo Pfu, according to the manufacturer's instructions, the product of PCR is a linear full-length

sequence containing the sequence of pBluescript with the sequence of mutated gene inserted.

4.The restriction enzyme Dpn I is then added to digest the template.

5.The mutated gene is excised from pBluescript and ligated into pET24a using standard molecular biology methods well known to those skilled in the art.

The plasmids carrying the mutant bOBP are transformed into the *E.coli* strain BL21(DE3) that are then grown on a sterile solid medium (LB Agar) containing the antibiotic kanamycin such that only those cells that have the plasmid in them can grow by virtue of their resistance to this antibiotic. A single colony is picked and cultured in a 25 ml shake flask containing 6 mls of LB broth containing 55µg/ml kanamycin, in an incubator (temperature 37°C, shaking speed 220rpm). This primary culture is transferred to a 2500 ml flask containing 1000 mls of LB broth containing 50µg/ml kanamycin, in an incubator (temperature 37°C, shaking speed 220rpm). Bacterial cells are collected by centrifugation and lysed by the use of a French Press. The clarified supernatant is passed through a nickel chelate column (HisBind™, Qiagen Inc.) and the bOBP eluted with 600 mM imidazole. The purified protein is labeled with acrylodans under the conditions according to the Molecular Probes protocol: bOBP is labeled with a 3:1 mole ratio of Acrylodans and left at room temperature for 30 minutes.

Example 4- Immobilisation of Site Specifically Labeled Binding Proteins onto Microscope Slides

A nucleotide sequence encoding the bovine odorant binding protein (bOBP) and carrying silent mutations that optimise codon usage in the expression host (*Escherichia coli*) is cloned in to the expression vector pET24a using techniques well known to those skilled in the art. The sequence of the gene is shown as SEQ.ID.No.1.

Mutations are made at positions 24, 36, 83 and 89 in the protein sequence using the technique of polymerase chain reaction according to the method given in Example 3 above. The mutations replace the natural residues with cysteine residues at these positions.

The plasmids carrying the mutant bOBP are transformed into the *E.coli* strain BL21(DE3) and then grown on a sterile solid medium (LB Agar) containing the antibiotic kanamycin such that only those cells which have the plasmid in them can grow by virtue of their resistance to this antibiotic. A single colony is picked and cultured in a 25 ml shake flask containing 6 mls of LB broth containing 50µg/ml kanamycin, in an incubator (temperature 37°C, shaking speed 220rpm). This primary culture is transferred to a 2500 ml flask containing 1000 mls of LB broth containing 50µg/ml kanamycin, in an incubator (temperature 37°C, shaking speed 220rpm). Bacterial cells are collected by centrifugation and lysed by the use of a French Press. The clarified supernatant is passed through a nickel chelate column (HisBind™, Qiagen Inc.) and the bOBP eluted with 600 mM imidazole. The purified protein is labeled with acrylodans under the conditions according to the Molecular Probes protocol: bOBP is labeled with a 3:1 mole ratio of Acrylodans and left for 30minutes at

room temperature.

Nickel nitrilotriacetate modified microscope slides from Xenopore Inc. are spotted with 5ul spots of solutions of bOBP (C36), bOBP (C39), each protein labeled with acrylodans as set out above.

Example 5- Screening Site Specific Cysteine Mutants Of Odorant Binding Protein For Ligand Dependent Changes in the Fluorescence of an Extrinsic Fluorophore

A nucleotide sequence encoding the bovine odorant binding protein (bOBP) and carrying silent mutations that optimise codon usage in the expression host (*Escherichia coli*) is cloned in to the expression vector pET24a using techniques described in Example 3 above.

Mutations are made at positions 24, 36, 83 and 89 in the protein sequence using the technique of polymerase chain reaction according to the methods described above. These mutations replace the natural residues with cysteine residues at the named positions.

The plasmids carrying the wild type or mutant bOBP are transformed into the *E.coli* strain BL21(DE3) which is then grown on a sterile solid medium (LB Agar) containing the antibiotic kanamycin such that only those cells that have the plasmid in them can grow by virtue of their resistance to this antibiotic.

Single colonies are then picked from the solid medium and added to 200ul of sterile LB medium (also containing kanamycin, LB^K) in the wells of a 96-well microplate. The plates are incubated at 37° C overnight with shaking.

20ul of this solution are then transferred to a fresh 200ul of LB^K also in a microplate well. A further 6 wells in the same plate are similarly prepared.

The plate is sealed with a plastic film and placed at 37° C with shaking for 1 hour. The sealing film is removed and 1 ul of a 1M solution of β-isopropyl thiogalactoside (IPTG) is added to each well, the plate resealed and incubated for further 4 hours.

The sealing film is removed and 100ul of BugBuster™ is then added to each well, the plate is resealed and incubated at room temperature for 30 minutes followed by centrifugation for 30mins at 4000rpm.

The contents of each well are then transferred to individual wells of a 96-well plate, which has been modified with nickel nitrilotriacetate groups (NiNTA) (Qiagen). The plate is sealed and incubated at room temperature for 1 hour. Liquid is then aspirated from the wells and each well washed 4 times with phosphate buffered saline (PBS).

After the last wash 200ul of PBS is added to each well followed by 1 ul of a 5mM solution of acrylodans.

The plate is then incubated for 30 minutes and washed 3 times with PBS. The wells are then filled with 200ul of PBS and the fluorescence emission spectrum measured for each well with excitation at 360nm.

1ul of a solution of the ligand (9mM thymol. dissolved in dimethylformamide) is then added into each well and the fluorescence emission spectrum again measured with identical instrument settings.

Spectra for the C24 mutant treated in this fashion are shown in Figure 2 with and without added ligands (thymol and menthol, concentrations as above).

Example 6: Discrimination between different ligands of bOBP with different variants of the protein.

Four different cysteine mutants of bOBP are made by the method described in Example 3. Each mutant has a cysteine residue introduced at a different position (one each of positions 24,36,83,89) and is subsequently labelled with acrylodans as described in Example 3. The labelled mutant proteins are then individually immobilised on separate nickel NTA microscope slides as described in Example 4. The slides are then cut into pieces and each piece placed in the wells of a 96 well microplate. Each column in the plate corresponds to a different variant. In each well one of 4 different samples is added (buffer, menthol, isomenthol, thymol) such that each row corresponds to a different sample. The well is then scanned such that the fluorescence intensity (λex 350, λem 400-600nm) is measured at 9 different positions in each well. The average intensity in each is then calculated.

Figure 3 shows the pattern of intensities for each ligand normalised to the signal in buffer for each protein.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

References

Chu, F.W., P.R. Edwards *et al.* (1997). "Microarray-based immunoassays". Acs Symposium Series **657**: 170-184.

Ekins, R. (1996). "Microspot(R), an array based ligand assay - Is this the ultimate microanalytical technology?" Journal of Clinical Ligand Assay **19**(2); 145-156.

Ekins,R., F. Chu, *et al.* (1990). "Multispot, multianalyte immunoassay." Annales De Biologie Clinique **48**(9): 655-666.

Ekins, R.P., F.W. Chu, *et al.* (1990). "Multianalyte immunoassay – The immunological compact-disk of the future." Journal of Clinical Immunoassay **13**(4): 169-181.

CLAIMS

1. A method for analysing a sample which method comprises:

(i) contacting the sample with a detector array comprising a plurality of discrete biological sensing elements; wherein each discrete biological sensing element comprises a detectable label whose characteristics change detectably when the element binds to a ligand within the sample and wherein the biological sensing elements are capable of binding more than one different ligand;

(ii) measuring the characteristics of the detectable label for each element of the array to produce a pattern; and

(iii) performing data analysis of the pattern.

2. A method according to claim 1 wherein the sample is liquid or substantially gaseous.

3. A method according to claim 1 or 2 wherein the detectable label is a fluorescent label.

4. A method according to any one of claims 1 to 3 wherein the change in the characteristics of the label is detected by optical or electrical means.

5. A method according to any one of the preceding claims wherein the biological sensing element is a protein selected from bacterial periplasmic binding proteins, membrane proteins, odorant binding proteins from mammalian or insect olfactory organs and DNA binding proteins.

6. A method according to any one of the preceding claims wherein the ligand(s) comprise volatile compounds.

7. A method according to any one of the preceding claims wherein step (iii) comprises either comparing the pattern obtained in step (ii) with a database of patterns from reference samples or forming a database of referemce patterns.

8. A detector array comprising a plurality of discrete biological sensing elements immobilised onto or within a solid support wherein each discrete biological sensing element comprises a detectable label whose characteristics change detectably when the element binds to a ligand within the sample; and wherein the sensing elements are provided in groups, each group comprising a biological sensing element and at least one variant of said element, said variant differing from the element in its ligand binding specificity and/or affinity.

9. An array according to claim 8 wherein the biological sensing elements are proteins selected from bacterial periplasmic binding proteins, membrane proteins, odorant binding proteins from mammalian or insect olfactory organs and DNA binding proteins.

10. An array according to claim 8 or 9 wherein the variant is derived from one of the other biological sensing elements by chemical modification.

11. An array according to claim 8 or 9 wherein the variant is derived from one of the other biological sensing elements by mutagenesis.

12. An array according to claim 11 wherein the mutagenesis is carried out by site-directed mutagenesis of a ligand binding site.

13. An array according to claim 11 wherein the mutagenesis is carried out by replacing a fragment of the biological sensing element polypeptide with a different polypeptide fragment.

14. An array according to claim 11 wherein the mutagenesis is random mutagenesis of the coding sequence.

15. Use of an array according to any one of claims 8 to 13 in a method of detecting bacteria in a liquid or substantially gaseous sample.

16. Use of an array according to any one of claims 8 to 13 in a method of detecting volatile compounds in a substantially gaseous sample.

17. A method of fingerprinting a sample comprising determining the activity profile of a sample against two or more different entities wherein said entities have a broad specificity binding activity.

18. A fingerprint obtained by the method of claim 17.

19. A sample when analysed either by the method according to claim 1 or any claim dependent thereon or by the method according to claim 17.

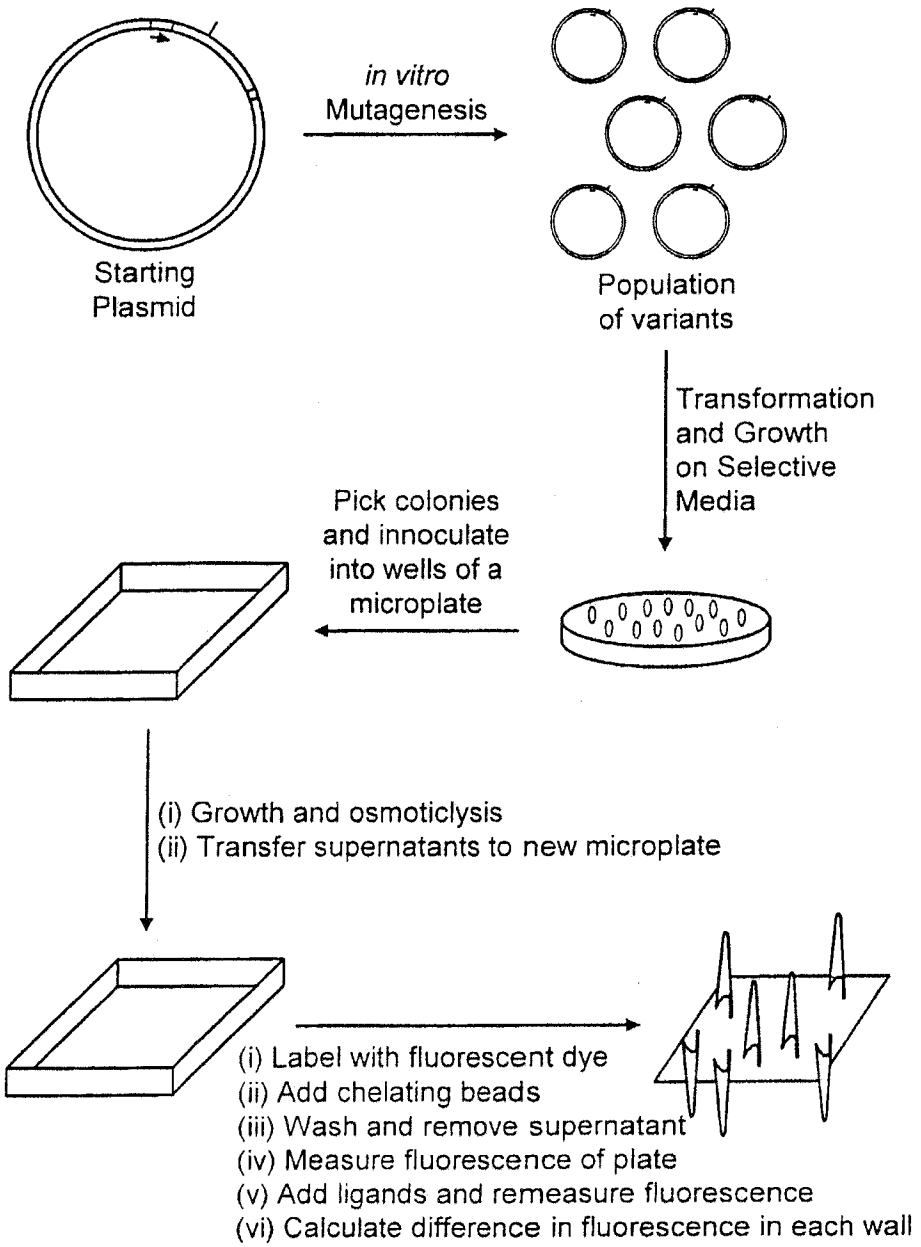
in vitro
Mutagenesis
Starting
Plasmid
Population
of variants
Transformation
and Growth
on Selective
Media
Pick colonies
and innoculate
into wells of a
microplate
(i) Growth and osmoticlysis
(ii) Transfer supernatants to new microplate
(i) Label with fluorescent dye
(ii) Add chelating beads
(iii) Wash and remove supernatant
(iv) Measure fluorescence of plate
(v) Add ligands and remeasure fluorescence
(vi) Calculate difference in fluorescence in each wall

FIG. 1

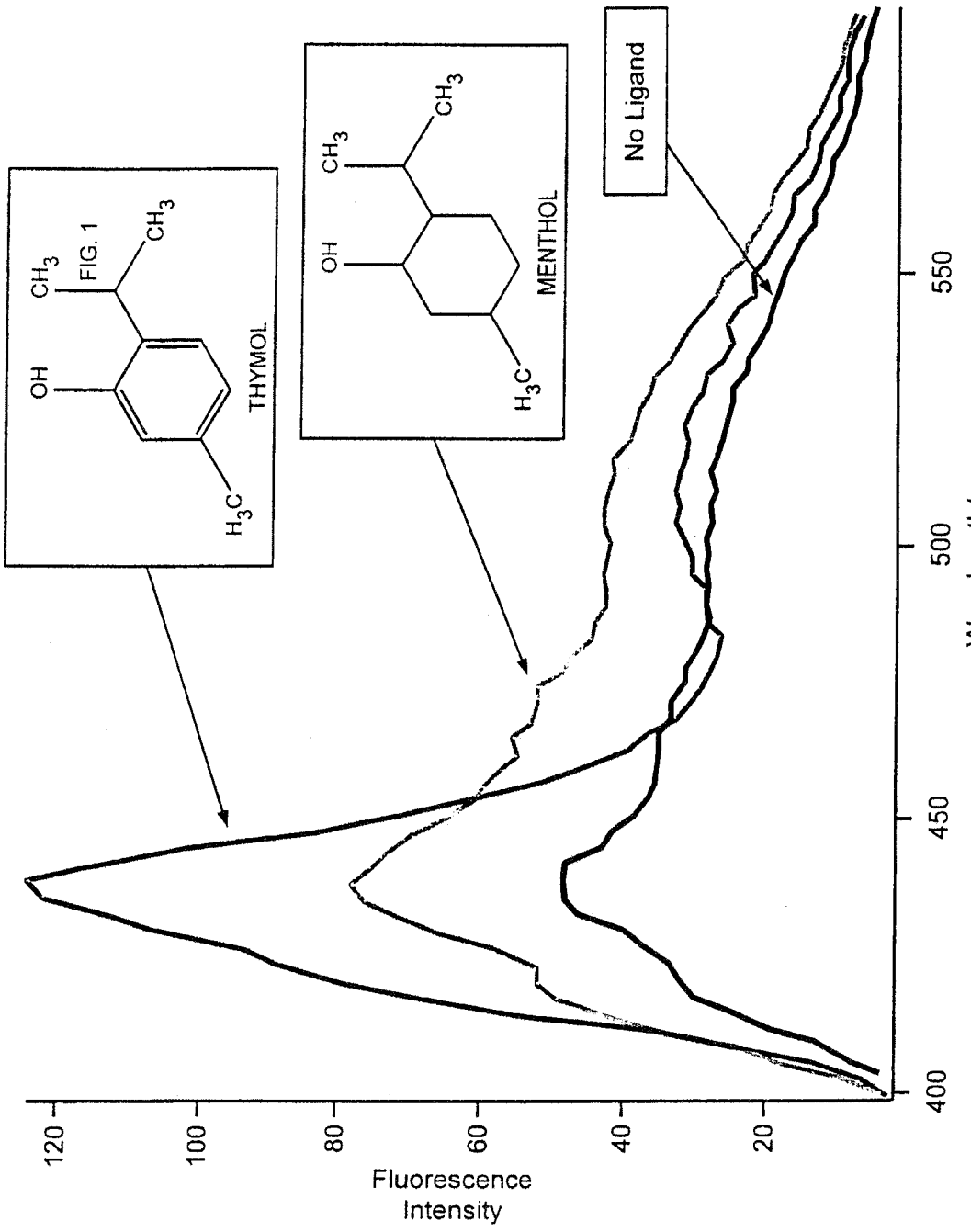
THYMOL
MENTHOL
No Ligand
Fluorescence Intensity
Wavelength/nm
120
100
80
60
40
20
400
450
500
550

FIG. 2

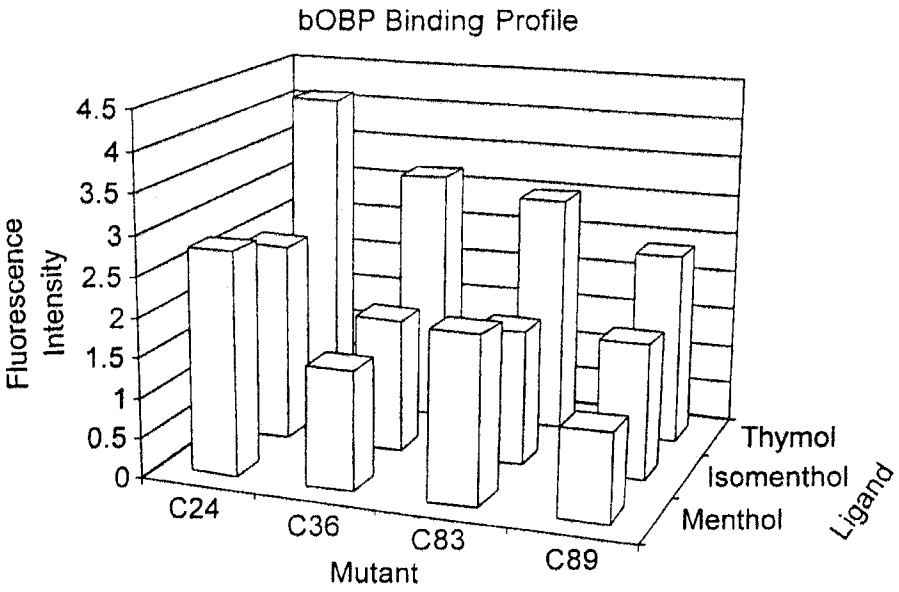
bOBP Binding Profile
Fluorescence Intensity
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
C24
C36
C83
C89
Mutant
Thymol
Isomenthol
Menthol
Ligand

FIG. 3

Sequence Listing

SEQ.ID.No.1

GGATCCTGGG	CGCAAGAGAG	AGGAGGAAAG	CTGAGCAAAA	TCTCTCAGAG	50
CTTTCAGGAC	CATGGAGAAC	AGTGTACATT	GGGTCGACCA	ACCCAGAGAA	100
AATCCAGGAG	AATGGACCAT	TCAGGACTTA	CTTCCGTGAA	CTTGTGTTTG	150
ATGATGAAAA	GGGCACGGTG	GACTTTTACT	TTTCTGTCAA	GCGGGATGGA	200
AAATGGAAGA	ATGTACATGT	CAAGGCTACA	AAGCAAGACG	ATGGTACTTA	250
TGTTGCTGAC	TATGAGGGTC	AAAACGTTTT	TAAAATTGTC	TCTCTGTCGA	300
GGACGCATCT	GGTAGCACAT	AACATCAACG	TGGATAAGCA	CAGCCAGAAG	350
ACAGAATTGG	CCGGACTGTT	TGTTAAACTG	AATGTTGAAG	ATGAAGACTT	400
GGAGAAATTC	TGGAAGCTGA	CGGAAGACAA	AGGAATTGAC	AAGAAAAACG	450
TTGTGAATTT	CTTGGAAAAT	GAAAACCATC	CCCACCCTGA	ACATCATCAT	500
CATCATCATC	ATCATCATCA	TTAG			

SEQ.ID.No.2

sequence of bOBP with 10X- His tag

1 ggatcctggg cgcaagagga ggaagctgag caaaatctct cagagctttc aggaccatgg
61 agaacagtgt acattggatc caccaaccca gagaaaatcc aggagaatgg accattcagg
121 acttacttcc gtgaacttgt gtttgatgat gaaaagggca cagtggactt ttacttttct
181 gtcaagcggg atggaaaatg gaagaatgta catgtcaagg ctacaaagca agacgatggt
241 acttatgttg ctgactatga gggtcaaaat gtatttaaaa ttgtctctct gtcgaggacg
301 catctggtag cacataacat caacgtggat aagcacggcc agaagacaga attgaccgga
361 ctgtttgtta aactgaatgt tgaagatgaa gacttggaga aattctggaa gctgacggaa
421 gacaaaggaa ttgacaagaa aaacgttgtg aatttcttgg aaaatgaaaa ccatccccac
481 cctgaacatc atcatcatca tcatcatcat catcattagg aattc

INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/GB 00/03768

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/533 G01N33/531

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHARYCH D H ET AL: "DIRECT COLORIMETRIC DETECTION OF A RECEPTOR-LIGAND INTERACTION BY A POLYMERIZED BILAYER ASSEMBLY" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 261, 1 July 1993 (1993-07-01), pages 585-588, XP002911062 ISSN: 0036-8075 the whole document ---	1,8
A	WO 97 49989 A (INTERACTIVA BIOTECHNOLOGIE GMB ;MECKLENBURG MICHAEL (SE); DANIELSS) 31 December 1997 (1997-12-31) cited in the application page 3, line 14 -page 4, line 25; examples 1,2 --- -/--	1-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 February 2001	07/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hart-Davis, J

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/GB 00/03768

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DAL MONTE MASSIMO ET AL: "Binding of selected odorants to bovine and porcine odorant-binding proteins." CHEMICAL SENSES, vol. 18, no. 6, 1993, pages 713-721, XP000986546 ISSN: 0379-864X the whole document	1-16
P,A	WO 99 51777 A (BUNSEN RUSH LAB INC ;LERNER MICHAEL R (US)) 14 October 1999 (1999-10-14) the whole document	1-16
T	TEGONI MARIELLA ET AL: "Mammalian odorant binding proteins." BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1482, no. 1-2, 2000, pages 229-240, XP000986537 ISSN: 0006-3002 the whole document	1-16
A	EP 0 844 246 A (SS PHARMACEUTICAL CO) 27 May 1998 (1998-05-27) abstract	1,3,4

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 17-19

Present claim 17 relates to an extremely large number of possible methods, and contains technical features of extremely broad scope ("sample", "entities"), these technical features not constituting a set of features sufficiently clear to enable the execution of a meaningful search. In fact, such a large number of assay methods could fall within claim 17 that a lack of clarity within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claim impossible.

Present claim 18, formally dependent on claim 17, which is in itself so unclear as to preclude the completion of a meaningful search, refers to a presentation of information (a "fingerprint"), which, following Rule 39 (v) PCT, constitutes subject matter which no International Searching Authority is required to search, following Article 17(2)(a)(i).

Present claim 19 refers to any sample analysed by the methods of claims 1 or 17 or claims dependent on claim 1. Any sample of any kind could in principle be analysed by the method of claim 1. Even if the search were to be restricted to compounds used as analytes in the examples of the application, such as menthol, isomenthol and thymol, these are known compounds, and cannot be considered as having novelty conferred upon by them by virtue of being subjected to a method of analysis. No definition of the subject matter for which protection is sought is therefore derivable from claim 19 (Article 6 PCT) and it has not been searched.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir ational Application No
PCT/GB 00/03768

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9749989 A	31-12-1997	AU 3436397 A	14-01-1998
		CA 2258941 A	31-12-1997
		EP 1021713 A	26-07-2000
		JP 2000513436 T	10-10-2000
		SE 9602545 A	26-12-1997
WO 9951777 A	14-10-1999	EP 1070257 A	24-01-2001
EP 0844246 A	27-05-1998	JP 9249662 A	22-09-1997
		JP 9249663 A	22-09-1997
		CA 2221306 A	18-09-1997
		WO 9733884 A	18-09-1997